application, it is over 28 times more prevalent in breast tissue than in the rest of the body. (Data obtained from the Lifeseq database developed by Incyte).

As is known to scientists skilled in the cancer diagnostic arts, few, if any circulating tumor associated antigens (TAA) are cancer specific (Stearns, V., et al., *Circulating Tumor Markers in Breast Cancer: Accepted Utilities and Novel Prospects,* Breast Cancer Research and Treatment 52: 239-59, 1998.) making diagnosis and screening difficult, if not impossible. Thus, the fact that a polynucleotide or protein is more prevalent in one tissue type than another means that it can be extremely useful as a cancer marker specific for that tissue. → can be - have not shown that it is — invitation to experiment

Proteins that are tissue-specific are important to cancer diagnostics because diseases such as cancer cause alterations to the host tissue resulting in the tissue-specific protein escaping from its normal compartment into a compartment where the protein is normally absent or is present at very low concentrations, such as blood or serum. Thus, if a protein appears in a tissue or body compartment where its normal occurrence is very low or non-existent, it indicates that the specific tissue in which the protein is normally found is in a diseased state. There are three main conditions which cause a tissue-specific protein to exist outside its specific host tissue: massive trauma, ischemia and hypertrophic proliferation. Thus, if a patient has not experienced a massive trauma or ischemia, detection of a tissue-specific protein outside that protein's host tissue indicates that the precise disease is hypertrophic proliferation of that tissue, the most serious form being cancer.

There are many examples of the diagnostic use of tissue-specific protein markers. Two examples are prostate specific antigen (PSA) and carcinoembryonic antigen (CEA). PSA is produced by the prostatic epithelium and the epithelial lining of the periurethral glands normally appearing in seminal plasma at high concentrations (Polascik, T.J., et al., *Prostate Specific Antigen: A Decade of Discovery – What We have Learned and Where We Are Going* J. Urology 162: 293-306, 1999). Thus, PSA itself is therefore characterized as specific to the normal prostate gland. In other words, PSA found in the prostate itself does not indicate a proliferative disease, such as cancer.

However, men with prostate cancer have elevated PSA levels in their circulation which is due to increased leakage from the luminal prostatic wall caused by the tumor growth (Brawer, M.K., *Prostate Specific Antigen.* Seminars in Surgical Oncology 18: 3-9, 2000.). An elevated PSA level in blood is indicative of prostate cancer and

have not shown the for BSA 794

consequently PSA has become the "most useful tumor marker in clinical practice" (Crawford, E.D., *The Utility of Prostate-Specific Antigen as a Surrogate Marker for Identification of High Risk Cohorts and Assessing Response in Chemoprevention Trials*. European Urology 35: 511-14, 1999) This is true even though PSA is found in the prostate of normal men. Thus, it is the tissue specificity of this marker itself which makes it diagnostically valuable. → have not linked tissue specificity w/ disease

Likewise, the appearance of carcinoembryonic antigen (CEA) in stool is normal, but its detection in blood at elevated levels is indicative of colorectal cancer. CEA is produced daily in substantial amounts by the normal colonic mucosa and in lesser amounts by mucous neck cells, stomach, tongue, esophagus, cervix, sweat glands and prostate (Hammarstrom S., *The Carcinoembryonic Antigen (CEA) Family: Structures, Suggested Functions and Expression in Normal and Malignant Tissues,* Seminars in Cancer Biology 9: 67-81, 1999).

In the normal person, CEA level in the blood is very low because colonic CEA is not in contact with the circulation and disappears with the stool. Colon cancer, however, causes abnormal changes in the cells and tissue of the colon resulting in the shedding of CEA into lymph and blood vessels (Hammarstrom, supra) The attached Exhibit A illustrates the usefulness of tissue-specific molecules which, upon detection in circulation, indicate proliferative disease. For example, Exhibit A states that CEA is expressed in normal adult tissue but is detected in serum in patients with colorectal and other carcinomas. (p. 67, col. 2). This journal article explains how a tissue specific molecule, expressed in the colon in normal individuals, is drained into lymph and blood vessels upon colon tumor growth. (Fig. 5). Consequently, CEA blood levels rise above normal levels which is clinically useful in monitoring colon cancer patients.

Clearly, the appearance of a secreted BS274 gene product outside the breast tissue itself, such as in whole blood, urine, stool or serum, indicates a form of breast disease, akin to the presence of common markers such as PSA and CEA found in blood outside of their prevalent tissue type. BS274's use in diagnostic test in order to determine whether a patient has a disease of the breast unquestionably illustrates a credible utility.

In addition to high tissue specificity, alone which makes BS274 a valuable cancer marker, this protein is *also* related to a family of proteins that are involved in malignant transformation. Specifically, this protein, a cytoskeleton-associated protein known as

CLIP-170, is a microtubule binding protein which is involved in the binding of endocytic vesicles to microtubules (Pierre, P., et al., *CLIP-170 Links Endocytic Vesicles to Microtubules*, Cell 70: 887-900, 1992; Perez, F., et al., *CLIP-170 Highlights – Growing Microtubule Ends in Vivo*, Cell 96: 517-527, 1999). This is significant due to the fact that malignant transformation involves abnormal changes in cellular structures involving the cytoskeleton (Holth, L.T., et al., *Chromatin, Nuclear Matrix and the Cytoskeleton: Role of Cell Structure in Neoplastic Transformation*, Int. J. Oncology 13: 827-37, 1998).

Specifically, CLIP-170 is the 90 – 180 amino acid region of BS274, with BS274 having 27% identity with the 115 – 200 amino acid region of CLIP-170 (Exhibit B). The 115 – 200 amino acid region of CLIP-170 is within the N-terminal domain which is defined as the 1 – 349 amino acid region. The complete CLIP-170 protein is composed of 1392 amino acids with an isoelectric point (pI) of 5.16. The N-terminal domain, however, is basic with a pI of 10.6 . BS174 is also basic with a theoretical pI of 9.6 which, along with its identity with CLIP-170, supports related functions between the two proteins.

The predicted secondary structure of the BS274 protein also has similar traits to that of CLIP-170. The N-terminal domain of CLIP-170 is characterized by turns and beta-sheets unlike the alpha-helical central domain defined as the amino acid region 350 – 1310 (Pierre, supra). When the 115 – 200 amino acid region of CLIP-170 was analyzed for secondary structure , four features were found to be present: 11.63% alpha helix, 59.3% extended strand, 12.79% beta turn, and 16.28% random coil. (Exhibit. C). The same analysis of the 90 – 180 amino acid region of BS274 resulted in the same four features, 21.98% alpha helix, 56.04% extended strand, 16.48% beta turn and 5.49% random coil (Exhibit. D). In both cases, the extended strand was the predominate characteristic between 56 and 60%. In contrast the 350 – 1310 amino acid region of CLIP-170 was 93.63% alpha helix (Exhibit E) agreeing with its description as an alpha-helical domain of 960 amino acids (Pierre, supra) .

CLIP-170 also has an alternatively spliced isoform known as restin. Restin has a 35 amino acid deletion in the alpha-helical central domain and, like CLIP-170, is also a microtubule-binding protein (Riehemann, K. and Sorg, C., *Sequence Homologies Between Four Cytoskeleton-Associated Proteins,* TIBS 18: 82-83, 1993; Scheel, J., et al., *Purification and Analysis of Authentic CLIP-170 and Recombinant Fragments*, J. Bio. Chem. 274: 25883-25891, 1999). The N-terminal domain of restin is therefore identical with that of CLIP-170 and has the same identity with BS274. It was noted that the N-terminal domain of

restin had several potential phosphorylation sites since the sequence contained motifs recognized by several kinases (Graeme Bilbe, et al., *Restin: A Novel Intermediate Filament-Associated Protein Highly Expressed in Reed-Sternberg Cells of Hodgkin's Disease*, The EMBO Journal 11: 2103-13, 1992). Likewise, these same motifs are in the BS274 sequence presenting six potential phosphorylation sites (Exhibit. F).

The aforementioned homology of BS-274 with the N- terminal region of CLIP-170 is significant due to the fact that the N-terminal region of CLIP-170/restin functions as the microtubule-binding domain in its interaction with the cell's cytoskeleton, which is related to neoplastic disease such as cancer. (Holth, L.T., et al., *Chromatin, Nuclear Matrix and the Cytoskeleton: Role of Cell Structure in Neoplastic Transformation*, Int. J. Oncology 13: 827-37, 1998.). Based on the key common characteristics and homology BS274 shares with the N-terminal domain of CLIP-170/restin, it is clear that BS274 functions as a cytoskeleton-associated protein.

The Examiner is reminded of the proper standard under the Revised Interim Utility Guidelines which specifically states that utility is acceptable if it is "believable to a person of ordinary skill in the art based on the totality of the evidence and reasoning provided". The Guidelines continue stating "[A]n assertion is credible unless (a) the logic underlying the assertion is **seriously** flawed, or (b) the facts upon which the assertion is based are inconsistent with the logic underlying the assertion", (emphasis added). Simply put, the threshold to be met by Applicant is a **credible assertion** of utility, not the extraordinarily high threshold improperly held by the Examiner.

Thus, BS 274's use as a diagnostic cancer marker, based on its tissue specificity as well as its strong homology to molecules long associated with cancer, is clearly evident. Thus it is respectfully requested that this rejection be withdrawn.

Claims 1-22, 38, 41, and 45-49 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, the Examiner alleges that the claimed invention is not supported by either a specific or substantial asserted utility or a well established utility for the reasons set forth above.

Based on the aforementioned arguments, it is respectfully requested that this rejection be withdrawn.

Claims 1-22, 38, 41, and 45-49 are rejected under 35 U.S.C. 112, first paragraph. The Examiner states that applicant's specification discloses a single BS274 gene sequence and a single BS274 protein sequence, yet Applicant's claims, which are to sequences having "at least 50% identity" with a few sequences taught in the specification, may encompass thousands of polynucleotides and that Applicant's definition of "% identity" is insufficient to provide a skilled artisan with the guidance necessary to clearly define the sequences encompassed by this claim language. Particularly, the Examiner states those having "at least 50% identity" with fragments of the sequences taught in the specification, would bear little resemblance to the single BS274 consensus sequence that Applicant has taught.

Therefore, Applicant submits the software manual to the Wisconsin Sequence Analysis program, Version 8, publicly available from Genetics Computer Group, Madison, WI, as Exhibit G. Support for this submission is found on page 13-14, beginning on line 35. The manual provides the algorithm, parameters, parameter values and other information necessary to, accurately and consistently, calculate the percent identity. This manual indicates on pages 5-21, *inter alia,* that the software used the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2; 482-489 (1981)). Additionally, Applicant has deleted "fragment" language and raised the percent identity substantially. Thus, it is respectfully requested that this rejection be withdrawn.

Claims 1-22, 38, 41, and 45-49 are rejected under 35 U.S.C. 112, first paragraph. Specifically, the Examiner states that it cannot be determined what is meant by polynucleotides "derived from" a BS274 polynucleotide", and that the claims also encompass fragments to BS274 polynucleotides.

Applicant has therefore deleted the "derived from" language and "fragment" language and it is respectfully requested that this rejection be withdrawn.

Claims 12-22, and 45-49 are said to be indefinite over the recitation of the phrase "derived from" in Claims 12 and 21. Therefore, Applicant has omitted this language from the claims and it is respectfully requested that this rejection be withdrawn.

Claim 13 is also rejected as indefinite over the recitation "hybridizes selectively". Thus, Applicant has deleted this language in an effort to expedite prosecution.

Claims 11-18, 45, and 46 rejected under 35 U.S.C. 102(b) as being anticipated by the following accession numbers:

Accession number AA266770 teaches a polynucleotide which has 70% sequence identity to a portion of SEQ ID NOS 2; accession number A21446 teaches a polynucleotide which has 77.8% sequence identity to a portion of SEQ ID NO 3; accession number A43962 teaches a polynucleotide which has 74.2% sequence identity to a portion of SEQ ID NO 4; accession number IL691 teaches a polynucleotide which has 71.9% sequence identity to a portion of SEQ ID NO 5; accession number I27772 teaches a polynucleotide which has 73.5% sequence identity to a portion of SEQ ID NO 6; and accession number I27772 teaches a polynucleotide that has 73.5% sequence identity to a portion of SEQ ID NO 7.

Due to the amendments which raise the percent identity, it is respectfully requested that this rejection be withdrawn.

CONCLUSION

In view of the aforementioned amendments and remarks, the aforementioned application is in condition for allowance and Applicant requests that the Examiner withdraw all outstanding objections and rejections and to pass this application to allowance.

Respectfully submitted,

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